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METABOLIC DISPOSITION OF LABELED WR-158,122 IN BILE-DUCT CANNUL--ETC(U)
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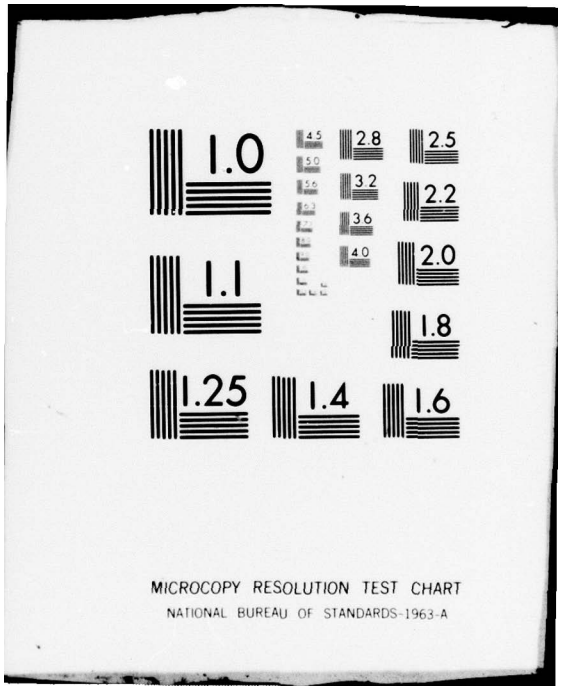
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(6) Metabolic Disposition of Labeled WR-158,122 in Bile-Duct Cannulated and Bile-Duct Ligated Rats and in a Monkey.

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sulfonylquinazoline	bile-duct cannulation
WR-158,122	bile-duct ligation
2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline	blood levels
rhesus monkey	excretion
rats	tissue distribution

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Blood levels, excretion, and tissue distribution of WR-158,122 ^{14}C were studied in one monkey, 7 bile-duct cannulated rats, 8 bile-duct ligated rats and 2 control rats. The monkey necropsied at 12 hours after dosing excreted 1.8% of the dose in urine and none in feces. It retained 93.6% of the dose in the gastrointestinal tract plus contents. The stomach contained 77.1 percent of the dose, and as a result there was very little drug and/or its metabolites in any other tissue. Blood and plasma peak level occurred at

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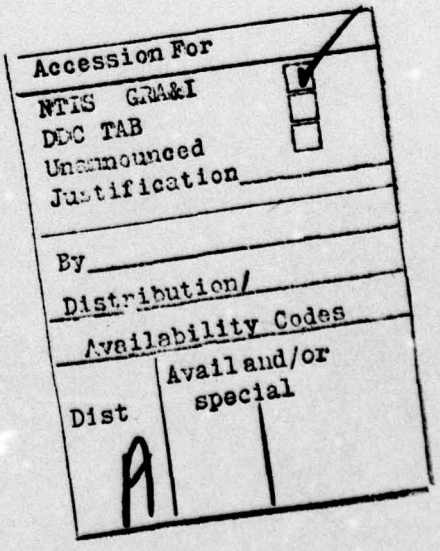
4 hours and plasma levels were higher than those in whole blood. Blood levels of bile-duct ligated rats were appreciably higher than the blood levels of bile-duct cannulated rats, but the blood curves peaked at the same time and followed the same general pattern. Bile-duct cannulated rats excreted 11.6-56.9 percent of WR-158,122 dose (as ^{14}C) in the bile. These rats generally excreted very little drug in the urine (4.5%). Bile-duct ligated rats excreted 13.5-36.3 percent of drug (as ^{14}C) in the urine. Feces are the main excretion route for WR-158,122 (as ^{14}C) in both bile-duct cannulated and bile-duct ligated rats accounting for 32.3 percent to 84.5 percent of the dose in the bile-duct cannulated rats and from 49.6 to 81.3 percent in the bile-duct ligated animals. Excretion of ^{14}C in both urine and feces was essentially complete at 48 hours. In both bile-duct cannulated and bile-duct ligated rats there is very little residual ^{14}C at 72 hours in liver, (heart, lungs, spleen, kidneys as a pool), gastrointestinal tract and contents, or carcass. Thus, in the rat it appears that WR-158,122 is rapidly, but imcompletely absorbed, transported to the liver and excreted in the bile.

TABLE OF CONTENTS

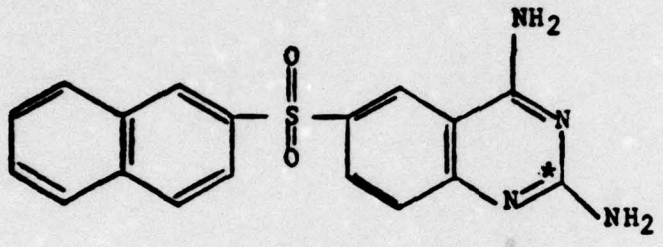


List of Tables

List of Figures

Interim Report No. 1

Studies on the metabolic fate of WR-158,122 [2,4-diamino-6-(2 naphthylsulfonyl)-quinazoline-2-^{14}C], the antifolate antimalarial compound shown below



*^{14}C

have been carried out in one rhesus monkey, 7 bile-duct cannulated rats, 8 bile-duct ligated rats and 2 control rats. Data on blood levels, excretion and tissue distribution are detailed in this report.

MATERIALS AND METHODS

Compounds. WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline-2-^{14}C], empirical formula $C_{18}H_{14}SO_2N_4$, mol. wt. 350, was supplied by Walter Reed unlabeled as Bottle AY 65859. The ^{14}C-labeled preparation was synthesized by Research Triangle Institute and dated 1/8/79. Lot No. 2572-110, dated 8/20/79, has a specific activity of 69 µCi/mg or 24 mCi/mmole. The radiochemical purity in a number of TLC systems was > 98%.

Treatment Suspension. The treatment suspension was prepared by grinding intimately 307.13 mg cold drug and 3.08 mg of ^{14}C labeled drug in a glass mortar with glass pestle with addition of small amounts of diluent (0.2% methylcellulose and 0.4% Tween 80 in distilled water) until a smooth suspension was achieved. The suspension was decanted into a tared round-bottom polycarbonate 200 ml centrifuge bottle containing glass beads and diluted to 124.08 g. It contained 2.50 mg WR-158,122/ml and 1.86 µCi/ml. The suspension was stored at 4°C. Assay of the suspension gave the following results:

4,136,000 dpm/ml
1654 dpm/µg
1.86 µCi/ml

Analytical Procedures

1. Blood. 0.2 or 0.3 ml of blood is digested with 0.5 ml of 0.5 N NaOH at 70°C. After 2 hours, 0.5 ml of t-butyl hydrogen peroxide (TBHP) is added and the sample is incubated for an additional 2 hours. After cooling the cocktail (15 ml of 4% DTN) is added followed by immediate vigorous shaking. After 2 hours cooling vials are counted in a Packard Tri Carb. All samples are corrected for quenching and other effects by internal standardization.

DTN Media

PPO Concentrate	Final Media
1 liter toluene	150 ml PPO concentrate is added to 3 l of dioxane
100 g PPO	Add 300 g naphthalene
2.5 g DiMePOPOP	Add 3.5 g BHT*
	Add 140 g Cab-O-Sil® (ca 4%)

* 2,6-Di-tert-butyl-p-cresol

2. Plasma. 0.5 ml of plasma is digested using the same procedure (eliminating the bleaching step) described for blood above.

3. Bile. Bile is counted exactly like blood except that the sample is usually 0.5 ml.

4. Urine. 0.5 ml samples of urine can usually be counted directly, but some highly-colored samples may require bleaching.

5. Feces. Feces are digested in appropriately sized jars with 10 volumes of 0.5 N NaOH for 48 hours at 70°C. Replicate 0.5 ml samples of the warm but not hot digest are bleached with 0.25 ml of TBHP for 2 hours and completed as above.

6. Tissues. Tissue samples in appropriately sized jars are digested with 4 volumes of 0.5 N NaOH at 70°C for 24 hours. Gastrointestinal tract and contents may require 48 hours. Replicate 0.5 ml or larger samples are bleached with TBHP 2 hours and counted as described above.

7. Carcass. Carcass is cut into several sections and a weight of H_2O equal to the carcass weight is added and 10 ml of 19 N NaOH are added for each 100 g of combined carcass and water. The digest after 24 hours is bleached and counted in the same way as the tissues described above. The bones do not digest in alkali but can be prepared for counting by dissolving them in an appropriate volume of 25% HNO_3 heated to about 90°C for several hours.

0.5 ml samples of this digest are counted in DTN-Cab-O-Sil. We found that for WR-158,122, the bones contained negligible amounts of ^{14}C activity.*

Monkey. One female rhesus monkey was placed in a chair approximately 24 hours before treatment using a small dose of Ketaset®. Water was available ad libitum. Control urine (216 ml) and control feces (23.8 g) were collected. The chair facilitated the collection of repeated blood samples as well as the quantitative collection of urine (in an iced container) and feces. The drug was administered by soft oral catheter as a single dose of 5 mg/kg. Blood samples were collected from the right or left antecubital vein at 2, 4, 6, 8, and 12 hours. At 12 hours the animal was given an anesthetic dose of Nembutal® and tissues were collected for distribution studies.

Rats. Sprague-Dawley-derived male rats weighing 245-376 g (bile-duct ligated) and 220 to 346 g (bile-duct cannulated) at time of surgery were used. The bile-duct ligated rats were housed in metabolism cages. The bile-duct cannulated rats were placed in wire restrainers attached to the metal cover of a plastic cage. Food and water were supplied ad libitum 4 hours after dosing. A bottle equipped with a funnel and a wire separator was positioned to collect all urine and feces. A tared vial was secured to the cage to collect bile. Blood samples were obtained at 2, 4, 6, 8, 12, 24, 48, and 72 hours from the tail vein in heparinized 200 µl pipettes. Bile, urine and feces were usually collected at 6, 12, 24, 48 and 72 hours. At 72 hours the rats were necropsied using an anesthetic dose of Nembutal®. Tissues assayed for ^{14}C included 1) liver; 2) heart, lungs, spleen and kidneys as a pool; 3) gastrointestinal tract and contents and 4) carcass.

*We are investigating other methods of measuring ^{14}C content of bone.

Bile-Duct Cannulation Procedure. The rats are fasted overnight and the following morning they are anesthetized with sodium pentobarbital (50 mg/kg i.p.). The abdomen is shaved and a transverse incision through the skin is made caudal to the last rib. The muscle layer is lifted, using forceps, and cut with blunt tip scissors being careful to avoid cutting the underlying tissues. The liver is retracted cranially using a warm saline-moistened gauze pad.

The area where the bile duct is cannulated is just caudal to the liver and superficial to the portal vein. Two ligatures are placed around the bile duct, the first caudal to the bifurcation of the bile duct and the second 5 mm caudal to the first ligature. The second ligature is tied causing the bile to build up in the bile duct. A small incision is made in the bile duct between the ligatures using a small scissors.

A polyethylene cannula (Clay-Adams PE 50), with one end cut at a 45 degree angle, is inserted into the bile duct and secured with the first and second ligature.

The cannula is exteriorized between the skin and muscle layer of the medial aspect of the left hind leg.

The muscle and skin layer of the abdomen are sutured separately and the skin is sealed with collodion.

When surgery is completed, the animal is placed in a stainless steel wire restrainer cage with the left hind leg secured to the restrainer using cotton cord with a ligature through the skin at the Achilles tendon (adapted from Robert E. Smyth, personal communication, 1977).

Bile-Duct Ligation Procedure. The rats are fasted overnight and the following morning they are anesthetized with sodium pentobarbital (50 mg/kg i.p.).

The abdomen is shaved and a transverse incision through the skin is made caudal to the last rib approximately 2½ cm in length. The muscle layer is lifted, using forceps, and cut with blunt tip scissors being careful to avoid cutting the underlying tissues. The liver is retracted cranially using a warm saline-moistened gauze pad.

The area where the bile duct is ligated is just caudal to the liver and superficial to the portal vein. Two ligatures of 4-0 silk are placed around the bile duct, the first caudal to the bifurcation of the bile duct and the second 5 mm caudal to the first ligature. Both ligatures are tied and the section of duct between the ligatures is cut with an iris scissors.

The muscle and skin layer of the abdomen are sutured separately and the skin is sealed with collodion.

RESULTS

A. Monkey. Blood Levels, Excretion and Tissue Distribution

Data on whole blood and plasma levels of WR-158,122 (as ^{14}C) were as follows:

Hours Post Dose	µg/g as ^{14}C	
	Blood	Plasma
2	0.18	0.25
4	0.43	0.62
6	0.40	0.58
8	0.33	0.49
12	0.36	0.46

Peak levels of radioactivity appeared in the blood and plasma at 4 hours. The plasma levels always exceeded the whole blood concentrations, findings which corroborate data on blood levels in rhesus monkeys submitted in Interim Report No. 40-1 on Contract No. DADA 17-67-C-7065 (March 16, 1972).

This monkey excreted 1.8% of the dose (as ^{14}C) in urine in 12 hours (see Table 1). No feces were excreted but the gastrointestinal tract and contents contained 93.6 percent of the dose (as ^{14}C) of which 77.1 percent was recovered in the stomach. There was very little localization of the compound (or its metabolites) in any tissue (Table 2), but this was probably due to the large amount of drug (^{14}C) still in the stomach.

B. Bile-Duct Cannulated Rats.

Blood Levels. The peak for blood levels varied widely ranging from 0.21 to 0.87 µg/g (as ^{14}C) (see Table 3 and Figure 1). Two rats had peak levels at 4 hours, four at 12 hours and one at 24. One rat (BC-6) showed a double peak at 12 hours and 48 hours.

Biliary Excretion. Total recovery of WR-158,122 and metabolites (as ^{14}C) in the bile by 72 hours ranged from 11.4 to 56.9 percent of dose (see Table 4 and Figure 2). This range is narrowed somewhat if BC-6 is eliminated. This animal is atypical in that it retained much more drug in the gut at necropsy time and eliminated much less in the feces than the other bile-duct cannulated rats. It also had an atypical double-peaked blood level curve. Peak levels of drug (as ^{14}C) in the bile occurred at 12 hours in two rats, at 24 hours in three rats, and at 48 hours in two animals. The peak for BC-1 might have occurred at a different time if bile flow had not been temporarily interrupted between 12 and 24 hours. In rat BC-2, the cannula became inoperative some time after the 48 hour collection and this accounts for the very small bile output at 72 hours. In rat BC-3 the cannula came out of the collection vial after 48 hours excluding the opportunity of collecting a 72 hour sample.

Urine Excretion. Total recovery of WR-158,122 (as ^{14}C) in the urine of bile-duct cannulated rats ranged from 0.4 to 15.6 percent of dose and is

essentially complete at 48 hours (see Table 4). As one would expect the range narrows to 0.4 to 8.5 percent if the data for BC-1 (a rat with temporary biliary stasis at 12-24 hours) is eliminated. The peak levels of drug as ^{14}C in the urine occurred at 24 hours in 4 rats and at 48 hours in the remaining animals.

Fecal Excretion. Excretion of drug and metabolite(s) in the feces was essentially complete in 48 hours. Total recovery in feces for the seven bile-duct cannulated rats ranged from 32.3-84.5 percent of dose (see Table 4). In rat BC-1, 50 percent of the dose was excreted in the 12-hour feces sample, but peak fecal excretion usually occurred at 24 hours.

Total Recovery. Total recovery varied from 87.2 to 114.7 percent of the dose with a mean of 101% (see Table 5). Fecal excretion accounted for an average of 65%, biliary excretion for 27% and urinary output for 4.5%. Except for the terminally atypical rat, BC-6, with apparent megacolon, the remaining radioactivity in all body compartments at 72 hours was usually less than 2%.

C. Bile-Duct Ligated Rats.

Blood Levels. As in the bile-duct cannulated rats, the peak blood levels varied widely (Table 6 and Figure 1). Three rats showed peaks at 12 hours, one rat at 24 hours and 2 rats at 48 hours. The unexpectedly high value for BL-6 at 48 hours (1.78 µg/g) may be an error due to some unrecognized contamination of the sample. It was not included in the average.

Urine Excretion. Total recovery of WR-158,122 (as ^{14}C) in the urine of bile-duct ligated rats ranged from 13.5 to 36.3 percent of dose (Table 7 and Figure 3). Excretion of drug in the urine was essentially complete at 48 hours. Peak urine excretion occurred at 12 hours for one rat, 24

hours for four rats, and at 48 hours for one animal.

Fecal Excretion. Excretion in the feces was essentially complete in 48 hours (see Table 7). Total recovery in feces for the six bile-duct ligated rats ranged from 49.6 to 81.3 percent. Peak fecal excretion occurred at 12 hours for one rat, at 24 hours for two rats, and at 48 hours for three rats.

Total Recovery. Total recovery ranged from 82.2 to 98.9 percent of the dose for the six bile-ligated rats with a mean of 89.4 percent (see Table 8). It is readily apparent that fecal excretion accounted for most of the drug. In all rats there was very little ^{14}C in the gastrointestinal tract and contents at 72 hours, and this was true for other tissues and carcass as well.

D. Control Rats Sacrificed at 24 Hours

In control rats sacrificed at 24 hours after dosing, 84-94 percent of the dose was recovered in feces (see Table 9). C-2 retained about 5 percent of the dose in the gut and contents and C-1 retained only 1.0 percent. Urinary excretion amounted to 2 to 5 percent and there was very little ^{14}C in the other tissues.

E. Bile-Duct Ligated Rats Sacrificed at 24 Hours.

In two bile-duct ligated rats sacrificed at 24 hours after dosing 43 to 57 percent of the dose was present in the feces. In both rats 11 percent of the dose was recovered in the urine and they retained 24 to 25 percent of the dose in the gastrointestinal tract and contents. Other organs (liver, heart, lungs, spleen, and kidneys) contained 1.2 to 1.5 percent of the dose and the carcass contained 6.4 and 8.8 percent of the dose as ^{14}C.

DISCUSSION

The data on the single 12-hour monkey appear to fit with our previous simian data collected at other time periods (see Report 40 of DADA 17-67-C-7065, 3/16/72).

From the data on biliary excretion in bile-duct cannulated rats it was concluded that WR-158,122 was partially absorbed and excreted in the bile. Two rats appeared to be atypical. In BC-1 the cannula closed temporarily between 12 and 24 hours and reopened some time between 24 and 48 hours which gave results mimicing a bile-duct ligated rat. Rat BC-6 retained feces so heavily in the gut that its condition bordered on megacolon. This rat retained 12.1 percent of the dose in gastrointestinal tract and contents at 72 hours whereas the other bile-duct cannulated rats retained less than 1 percent. This rat also excreted the largest amount of drug and/or metabolites in its bile (57%). Rat BC-A was a pilot experiment and the rat was appreciably heavier than the six other bile-duct cannulated rats; however, the data on this rat compare very well with the data of the six bile-duct cannulated rats run later except for higher blood level data (see Table 3).

When blood levels of bile-duct cannulated rats were compared with blood levels of bile-duct ligated rats it was apparent that levels for bile-duct cannulated rats were appreciably lower than levels for bile-duct ligated rats (Figure 1) but the peak blood levels for both groups occurred at 12 hours and the curves followed the same general pattern.

In our earlier work with control rats the peak blood levels of WR-158,122 (as ^{14}C) occurred at 3 hours after dosing (Interim Report No. 40-1) whereas in bile-duct cannulated and bile-duct ligated rats the peak levels usually occurred at 12 hours or later.

As one might anticipate bile-duct ligated rats excreted much more drug in the urine (20%) than bile-duct cannulated rats (4.5%). Fecal excretion of ^{14}C in bile-duct cannulated and bile-duct ligated rats was comparable. The one rat, BC-6, with low fecal excretion (32.3 percent of dose) had 56.9 percent of its dose in the bile and 12.1 percent of its dose in gastrointestinal tract plus contents. The one rat in the bile-duct ligated series (BL-5) with somewhat low fecal excretion (49.6 percent of dose) excreted the largest amount (36.3%) in the urine.

Except for BC-6 there was very little residual ^{14}C in gastrointestinal tract plus contents in either the bile-duct cannulated or the bile-duct ligated animals. It is interesting that the two bile-duct ligated rats sacrificed at 24 hours retained about 25 percent of the dose in the gut. Control rats on the other hand retained only 1-5 percent of the dose in gastrointestinal tract and contents at 24 hours.

At 72 hours both bile-duct cannulated and bile-duct ligated rats retained less than 1.0 percent of the dose in the other tissues which included liver, heart, lungs, spleen and kidneys.

The mean liver wt./body wt. ratio for bile-duct cannulated rats was 0.039 ± 0.004 and that for bile-duct ligated animals was 0.052 ± 0.002. Thus the mean liver weight for bile-duct cannulated rats was 9.3 g and for bile-duct ligated rats was 14.6 g. The liver of bile-duct cannulated rats contained 0.4% of the dose of ^{14}C on the average, whereas bile-duct ligated rats with larger livers contained only about one-third as much ^{14}C.

Carcass retained very little of the dose at 72 hours, usually less than 1%. In the two bile-duct ligated rats sacrificed at 24 hours the carcass retained 6 to 9 percent of the dose.

SUMMARY

1. Blood levels, excretion, and tissue distribution of WR-158,122 ^{14}C were studied in one monkey, 7 bile-duct cannulated rats, 8 bile-duct ligated rats and 2 control rats.

2. The monkey necropsied at 12 hours after dosing excreted 1.8% of the dose in urine and none in feces. It retained 93.6% of the dose in the gastrointestinal tract plus contents. The stomach contained 77.1 percent of the dose, and as a result there was very little drug and/or its metabolites in any other tissue. Blood and plasma peak level occurred at 4 hours and plasma levels were higher than those in whole blood.

3. Blood levels of bile-duct ligated rats were appreciably higher than the blood levels of bile-duct cannulated rats, but the blood curves peaked at the same time and followed the same general pattern.

4. Bile-duct cannulated rats excreted 11.6-56.9 percent of WR-158, 122 dose (as ^{14}C) in the bile. These rats generally excreted very little drug in the urine (4.5%).

5. Bile-duct ligated rats excreted 13.5-36.3 percent of drug (as ^{14}C) in the urine.

6. Feces are the main excretion route for WR-158,122 (as ^{14}C) in both bile-duct cannulated and bile-duct ligated rats accounting for 32.3 percent to 84.5 percent of the dose in the bile-duct cannulated rats and from 49.6 to 81.3 percent in the bile-duct ligated animals.

7. Excretion of ^{14}C in both urine and feces was essentially complete at 48 hours.

8. In both bile-duct cannulated and bile-duct ligated rats there is very little residual ^{14}C at 72 hours in liver, (heart, lungs, spleen,

kidneys as a pool), gastrointestinal tract and contents, or carcass.

9. Thus, in the rat it appears that WR-158,122 is rapidly, but incompletely absorbed, transported to the liver and excreted in the bile.

Table 1

Recovery of WR-158,122 from a Rhesus Monkey

Single Oral Dose of 5 mg/kg

	Percent of Dose Recovered as ^{14}C 12 Hours Post Dose
Urine	1.8
Feces	N.S.*
Gastrointestinal Tract & Contents	93.6
Other Tissues	2.0
Bile**	0.02 (1.36 g)
TOTAL	97.4

* No Sample

** The figure in parentheses represents the weight of bile in grams recovered from the gall bladder at time of necropsy.

Table 2

Tissue Distribution of WR-158,122 in a Rhesus Monkey

Single Oral Dose of 5 mg/kg

	Recovery (as ^{14}C) in Percent of Dose and µg/g 12 Hours Post Dose	
Tissue	Percent of Dose per Tissue	µg/g
Stomach*	77.1	58.2
Small Intestine*	0.76	2.8
Cecum*	2.4	13.4
Large Intestine*	13.3	17.8
Liver	0.43	0.9
Gall Bladder	<0.01	1.2
Bile	0.02	4.4
Lungs	0.10	0.5
Heart	0.07	1.3
Pancreas	0.04	0.8
Spleen	0.01	0.5
Adrenals	<0.01	0.5
Kidneys	0.13	1.0
Urinary Bladder	0.10	2.7
Muscle, Skeletal+	0.49	0.1
Whole Blood++	0.65	0.4
TOTAL	95.6	

* Tissue plus contents

+ Based on skeletal muscle weight equals 20% of necropsy body weight

++ Based on blood volume equivalent to 9% of necropsy body weight

Table 3

Blood Levels of WR158,122 in Bile-Duct Cannulated Rats

Single Oral Dose 10 mg/kg

	µg/g as ^{14}C						
Hours Post Dose	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A*
1							0.57
2	0.17	0.04	0.12	0.10	0.07	0.06	0.69
4	0.32	0.05	0.21	0.17	0.11	0.12	0.81
6	0.52	0.09	0.20	0.28	0.21	0.12	0.72
8	0.67	0.09	0.20	0.28	0.28	0.23	0.55
12	0.87	0.14	0.17	0.37	0.67	0.34	0.32
24	0.75	0.29	0.15	0.15	0.56	0.28	0.11
48	0.25	0.05	0.04	0.05	0.20	0.34	0.03
72	0.06	0.04	0.01	0.01	0.02	0.11	0.02
Hct (Terminal)	39	41	50	45	46	53	46

* BC-A weighed 480 g; the other rats ranged from 220 to 346 g.

Table 4

Excretion of WR-158,122 in Bile-Duct Cannulated Rats

Single Oral Dose 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C						
		BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A
Bile (g)	6	I.S.*	I.S.	6.8 (5.6)	4.4 (5.2)	1.3 (2.5)	2.4 (4.6)	I.S.
	12	11.8 (7.6)	4.8 (7.2)	7.5 (5.4)	8.5 (4.9)	4.2 (2.0)	8.3 (4.7)	8.9 (5.3)
	24	0.08 (5.0)	11.4 (7.4)	8.8 (9.7)	11.1 (9.0)	5.5 (6.9)	17.7 (9.2)	1.8 (7.3)
	48	0.17 (4.4)	21.2 (13.1)	5.6 (14.1)	3.4 (13.5)	3.4 (7.6)	23.2 (14.7)	0.5 (15.0)
	72	0.41 (23.6)	0.09 (1.1)	N.S.**	0.25 (12.5)	0.5 (21.1)	5.3 (14.0)	0.2 (10.9)
TOTAL		12.5	37.5	28.7	27.6	14.9	56.9	11.4
Urine (ml)	6	I.S.	I.S.	0.24 (5.6)	0.03 (0.8)	0.03 (3.9)	0.02 (1.1)	I.S.
	12	3.7 (6.0)	0.42 (3.5)	0.5 (12.0)	0.58 (2.4)	1.2 (4.4)	0.21 (1.6)	0.15 (3.0)
	24	9.9 (13.0)	1.6 (7.1)	0.9 (39.0)	1.3 (12.9)	4.6 (19.2)	2.8 (9.0)	N.S.**
	48	1.8 (21.6)	3.7 (15.2)	0.8 (45.5)	0.4 (24.5)	1.5 (25.3)	4.4 (27.0)	0.19 (19.6)
	72	0.24 (23.0)	0.23 (5.5)	0.05 (23.0)	0.04 (14.2)	0.11 (24.0)	1.1 (17.0)	0.04 (24.0)
TOTAL		15.6	6.0	2.5	2.4	7.4	8.5	0.4

Table 4 Continued

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C						
		BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A
Feces (g)	6	I.S.	I.S.	0.01 (1.5)	0.02 (4.5)	N.C.***	N.C.	I.S.
	12	49.9 (4.6)	21.3 (3.1)	16.2 (0.9)	0.01 (0.3)	N.C.	N.C.	28.7 (1.6)
	24	21.7 (2.8)	12.3 (6.2)	40.6 (9.3)	58.2 (4.7)	47.1 (2.0)	28.6 (6.0)	43.4 (3.0)
	48	12.7 (8.8)	21.5 (10.1)	5.9 (7.0)	8.6 (5.4)	27.5 (3.3)	1.5 (0.4)	0.03 (1.4)
	72	0.23 (5.3)	1.3 (1.2)	1.4 (3.7)	1.6 (4.7)	0.5 (5.1)	2.2 (0.6)	2.3 (0.02)
TOTAL		84.5	56.4	64.1	68.4	75.1	32.3	74.4

( ) - Number in parenthesis is sample size in g or ml.

* I.S. - Insufficient sample

** N.S. - No sample

*** N.C. - Sample not collected

Table 5

Recovery of WR-158,122 from Bile-Duct Cannulated Rats

Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 72 Hours						
	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-A*
Urine	15.6	6.0	2.5	2.4	7.4	8.5	0.4
Feces	84.5	56.4	64.1	68.4	75.1	32.3	74.3
Bile	12.5	37.5	28.7	27.6	14.9	56.9	11.6
Gastrointestinal Tract & Contents	0.5	0.6	0.07	0.03	0.02	12.1**	0.23
Other Tissues	0.9	0.7	0.2	0.2	0.4	0.5	0.3
Carcass	0.7	0.6	0.8	0.1	0.2	0.7	0.4
TOTAL	114.7	101.8	96.4	98.7	98.0	111.0	87.2

* BC-A weighed 480 g; the other rats weighed from 220 to 340 g.

** BC-6 - abnormal retention of feces bordering on megacolon.

Table 6

Blood Levels of WR-158,122 in Bile-Duct Ligated Rats
Single Oral Dose 10 mg/kg

Hours Post Dose	µg/g as ^{14}C					
	BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
2	0.25	0.32	0.18	0.10	0.14	0.28
4	0.45	0.57	0.28	0.13	0.34	0.53
6	0.63	0.72	0.53	0.19	0.43	0.59
8	0.81	0.90	0.69	0.34	0.57	0.77
12	0.85	1.12	0.86	0.46	0.70	0.86
24	0.38	0.52	0.40	0.45	1.16	0.81
48	0.19	0.22	0.12	0.50	0.48	1.78
72	0.07	0.06	0.04	0.14	0.21	0.13
Hct (Terminal)	40	39	49	47	45	43

Table 7

Excretion of WR-158,122 in Bile-Duct Ligated Rats
Single Oral Dose 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C					
		BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
Urine (ml)	6	0.04 (2.0)	0.9 (2.0)	0.37 (1.7)	0.66 (3.1)	0.56 (1.0)	0.03 (1.4)
	12	4.6 (9.3)	4.4 (9.5)	8.2 (4.1)	1.8 (1.5)	2.5 (1.2)	1.9 (1.5)
	24	4.9 (7.4)	6.3 (6.7)	6.1 (10.0)	5.0 (6.5)	15.6 (5.7)	7.5 (8.2)
	48	3.4 (16.2)	3.5 (20.6)	2.4 (11.8)	9.4 (6.6)	14.9 (11.2)	6.3 (12.0)
	72	0.51 (26.5)	0.39 (18.0)	0.25 (12.2)	1.5 (10.4)	2.7 (13.6)	2.4 (32.0)
TOTAL		13.5	15.5	17.3	18.4	36.3	18.1
Feces (g)	6	11.4 (2.9)	15.6 (3.8)	<0.01 (0.24)	<0.01 (0.13)	N.C.**	N.C.
	12	7.3 (0.26)	12.7 (0.68)	49.6 (3.8)	N.S.*	N.C.	N.C.
	24	21.7 (0.49)	37.5 (2.4)	30.0 (4.3)	14.0 (3.8)	11.3 (2.6)	50.5 (4.0)
	48	26.5 (3.5)	8.5 (4.1)	1.6 (4.3)	53.8 (10.3)	34.1 (5.9)	11.8 (4.4)
	72	1.4 (5.1)	0.62 (8.2)	0.14 (4.5)	1.6 (5.8)	4.2 (5.0)	1.0 (3.2)
TOTAL		68.3	74.9	81.3	69.4	49.6	63.3

( ) - Number in parenthesis is sample size in g or ml.

* N.S. - No sample

** N.C. - Sample not collected

Table 8

Recovery of WR-158,122 from Bile-Duct Ligated Rats
Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 72 Hours					
	BL-1	BL-2	BL-3	BL-4	BL-5	BL-6
Urine	13.5	15.5	17.3	18.4	36.3	18.1
Feces	68.3	74.9	81.3	69.4	49.6	63.3
Gastrointestinal Tract & contents	0.11	0.08	0.03	0.22	0.18	0.14
Other tissues	0.15	0.13	0.11	0.32	0.26	0.21
Carcass	0.35	0.52	0.15	0.53	0.58	0.48
TOTAL	82.4	91.1	98.9	88.9	86.9	82.2

Table 9

Recovery of WR-158,122 in Bile-Duct Ligated Rats and Control Rats (oral 10 mg/kg)

	Percent of Dose Recovered as ^{14}C 24 Hours After Treatment			
	BL-7	BL-8	C-1	C-2
Urine	10.7	11.2	1.6	4.5
Feces	56.7	42.5	93.9	84.0
Gastrointestinal Tract and Contents	24.7	29.6	1.0	4.9
Other Tissues	1.2	1.8	0.1	0.7
Carcass	4.8	6.4	0.1	0.4
TOTAL	97.9	91.5	96.7	94.5

Table 10

Recovery of WR158,122 in Bile-Duct Ligated and Control Rats (oral 10 mg/kg)

	µg/g Recovered as ^{14}C 24 Hours After Treatment			
	BL-7	BL-8	C-1	C-2
Gastrointestinal Tract and Contents	37.1	51.9	1.4	6.8
Liver	2.4	3.6	0.3	1.0
Lungs	1.0	2.1	<0.1	0.2
Heart	0.6	1.1	<0.1	<0.1
Spleen	0.7	1.2	<0.1	<0.1
Kidneys	2.4	5.0	0.2	0.4
Whole Blood	0.4	0.6	<0.1	<0.1
Carcass	0.2	0.3	<0.1	<0.1

Figure 1

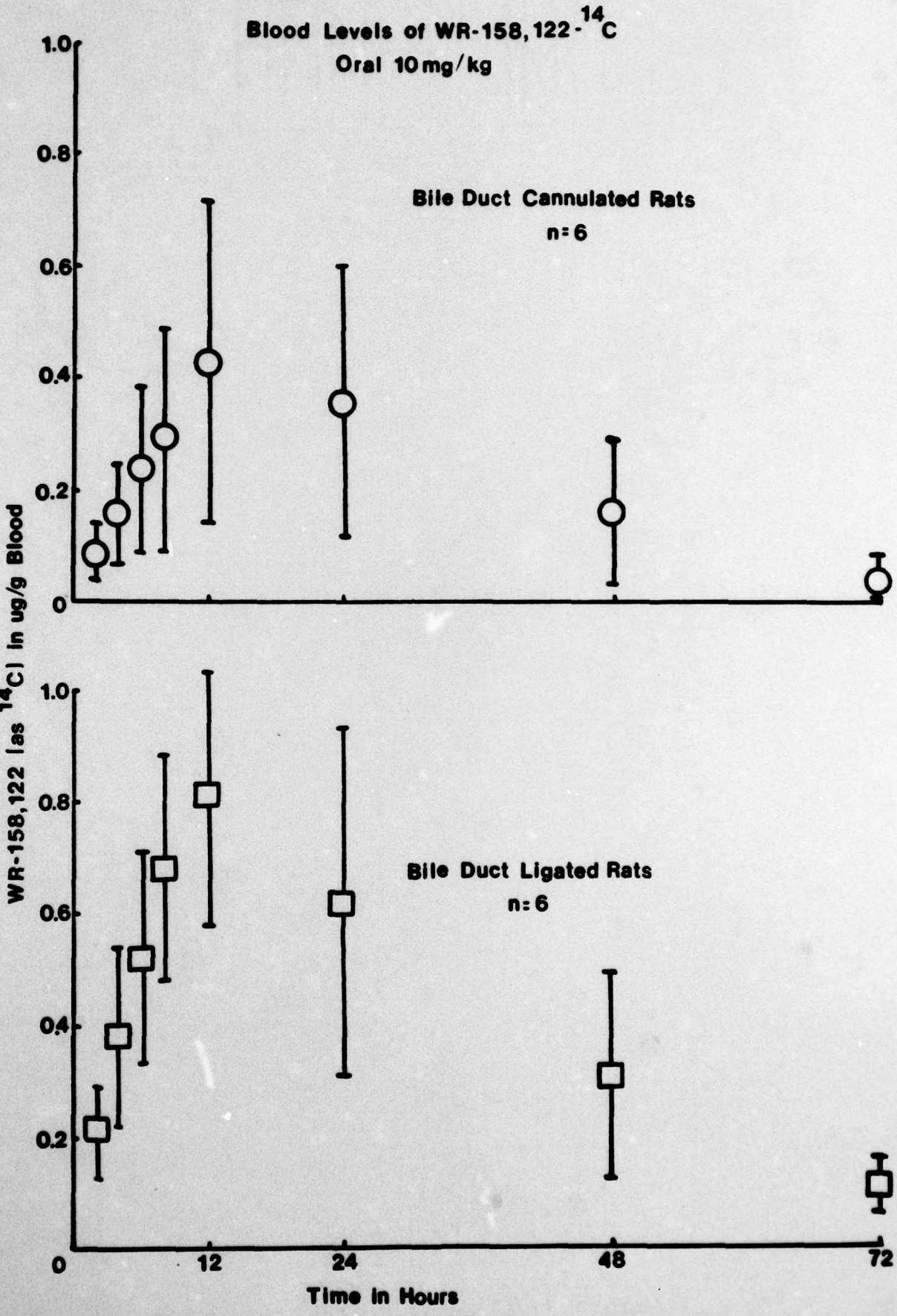

Blood Levels of WR-158,122-^{14}C
Oral 10 mg/kg
Bile Duct Cannulated Rats
n=6
Bile Duct Ligated Rats
n=6
WR-158,122 las ^{14}Cl in ug/g Blood
1.0
0.8
0.6
0.4
0.2
0
12
24
48
72
Time in Hours

Figure 2
Cumulative Excretion of WR-158,122

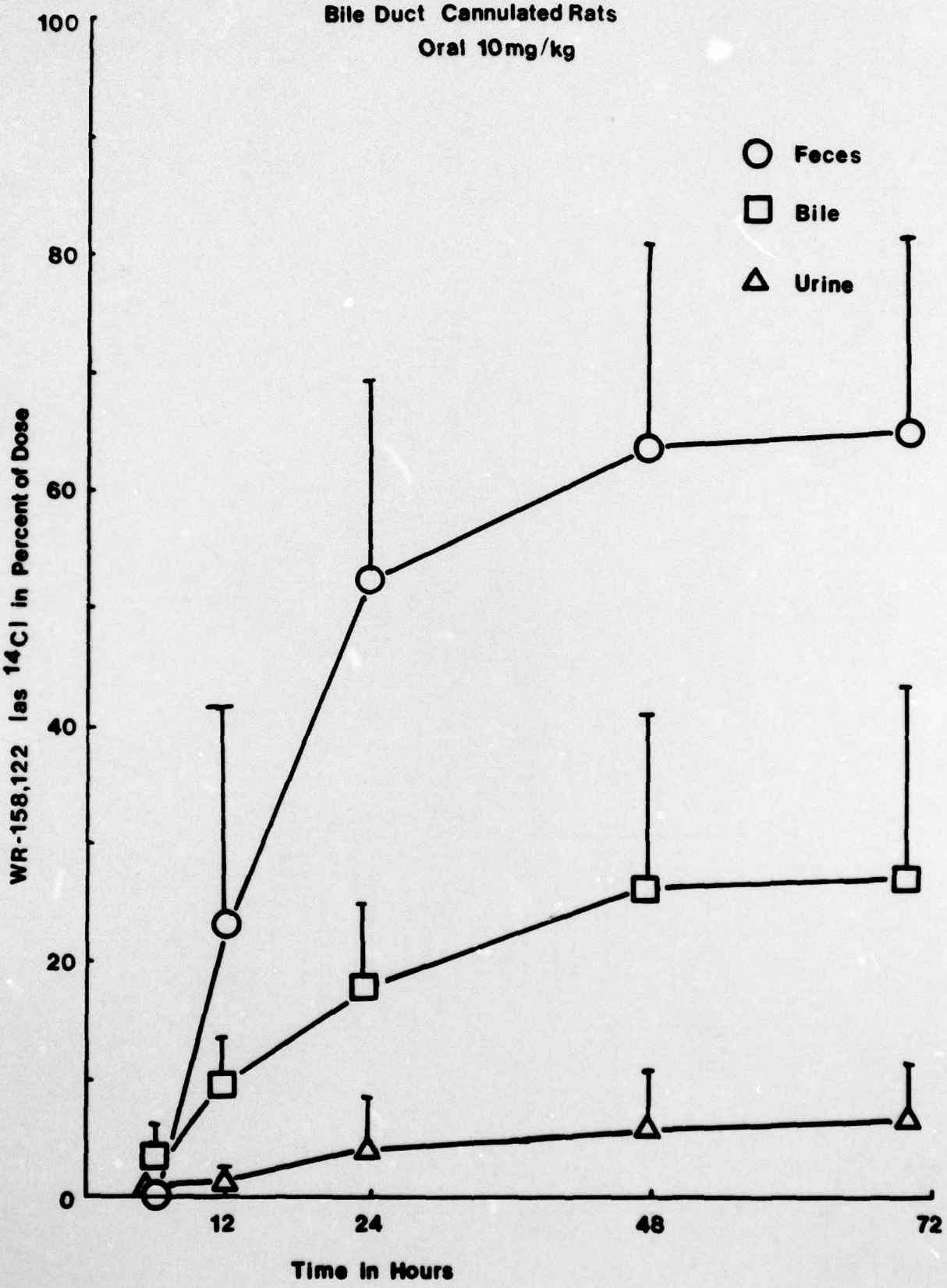
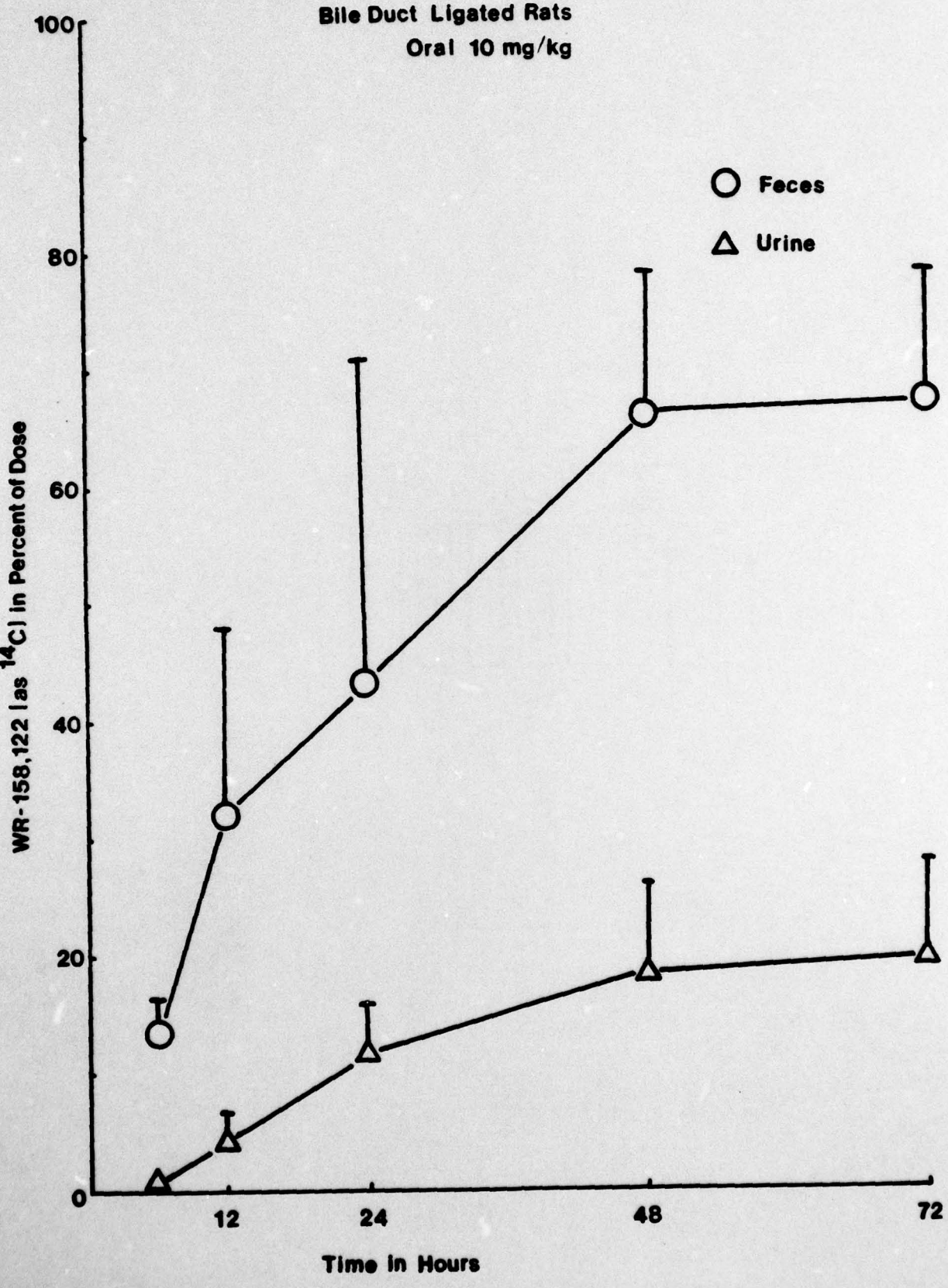


Figure 3

Cumulative Excretion of WR-158,122



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